

ORIGINAL ARTICLE

ASSOCIATION OF BODY MASS INDEX WITH
CLOPIDOGREL RESISTANCE

Usman Nawaz, Mudassar Noor, Chaudhry Altaf*, Akbar Waheed, Saleem Ahmad Khan**

Department of Pharmacology and Therapeutics, Army Medical College, National University of Medical Science, *Department of Haematology, Armed Forces Institute of Pathology, **Department of Pathology, Army Medical College, National University of Medical Science, Rawalpindi, Pakistan

Background: Clopidogrel is an essential component of dual antiplatelet treatment for ischemic heart disease. We planned to explore the association of body mass index with clopidogrel resistance in ischemic heart disease patients. **Methods:** Three hundred and ninety patients of ischemic heart disease who were taking clopidogrel 75mg/day, were included in the study. Patients were categorized according to their body mass index (BMI) into underweight (BMI<18.50 Kg/m²), normal weight (BMI 18.50–22.99 Kg/m²), overweight (BMI 23.00–24.99 Kg/m²), and obese (BMI>25 Kg/m²). Their blood samples were taken and platelet aggregation studies were performed using Chronolog light transmission aggregometer to confirm clopidogrel resistant status. **Results:** None of the underweight, 19.50% of the normal weight, 24.20% of the overweight and 33.60% of the obese patients were found to be clopidogrel resistant. Clopidogrel resistance was significantly higher in obese patients as compared to normal weight patients, $p=0.019$. No significant difference in clopidogrel response was found between normal weight and overweight patients, as well as overweight and obese patients. Mean BMI of clopidogrel resistant patients was significantly higher than mean BMI of clopidogrel responders (26.46±3.82 vs 24.15±3.39 Kg/m²), $p<0.001$. Correlation between BMI and platelet aggregation was significant ($p<0.001$, $r=0.20$). **Conclusion:** BMI is inversely related to the platelet inhibition by clopidogrel. Higher BMI is related to clopidogrel resistance in ischemic heart disease patients.

Keywords: BMI, clopidogrel responders, clopidogrel resistance, Pakistan

Pak J Physiol 2018;14(3):50–3

INTRODUCTION

Clopidogrel is used along with aspirin in dual antiplatelet regimen for the treatment of ischemic heart disease (IHD) as well as prophylaxis of secondary ischemic events after angina, myocardial infarction, or percutaneous coronary stenting. Secondary events are in the form of ischemia, re-infarction, occlusion of stents, and even death. Clopidogrel is an oral drug and belongs to thienopyridine group. It is a prodrug and activated inside body to active metabolites.^{1,2}

Adenine diphosphate (ADP) is secreted from activated platelets and it stimulates ADP receptors on the surface of the platelets, resulting in further platelet aggregation, which is primary step in formation of thrombi in coronary vasculature. Clopidogrel acts by antagonizing ADP P2Y₁₂ receptors, thus preventing activation of glycoprotein IIb/IIIa receptors and inhibiting platelet aggregation.¹

Several studies have demonstrated resistance to the clopidogrel antiplatelet action among different populations, resulting in lesser clinical efficacy of the drug. Several factors may contribute to clopidogrel resistance, which may be poor compliance, enhanced platelet turnover, obesity, hypertension, diabetes mellitus or dyslipidemia.³ Clopidogrel resistance is an independent predictor of secondary ischemic events in patients undergoing percutaneous coronary intervention with drug eluting stents.⁴

Genetic mutations can also cause clopidogrel resistance which may include variations in genes encoding CYP2C19 and CYP3A4 enzymes responsible for clopidogrel activation in liver, or in P2Y₁₂ ADP receptor gene that is target site of the drug.⁵ Some studies have indicated that high body mass index (BMI) is an independent predictor of platelet reactivity along with acute coronary syndrome, diabetes mellitus and CYP2C192 mutation.^{6,7} There are 77.8% chances of high platelet residual activity despite clopidogrel therapy in patients having anemia, diabetes mellitus and obesity. It has been documented that BMI greater than 28 Kg/m² is a risk factor for inadequate platelet inhibition in patients receiving clopidogrel in certain populations.² The current study was planned to find out association between BMI and clopidogrel resistance in IHD patients.

METHODOLOGY

This cross-sectional analytical study was conducted from April 2015 to May 2017 at Department of Pharmacology and Therapeutics, Army Medical College, Armed Forces Institute of Cardiology, and Armed Forces Institute of Pathology under the auspices of National University of Medical Sciences, Rawalpindi, Pakistan. The study was approved by Ethical Review Committee of the University.

Ischemic heart disease patients of either sex, with age over 15 years and taking clopidogrel 75

mg/day for at least 7 days with good compliance were included in the study after informed written consent. Patients with active liver or renal disease, pregnancy, malignancy, or taking anticoagulants like heparin or warfarin, or taking omeprazole were excluded from the study.⁸

Sample size was calculated to be 390 using WHO sample size calculator. A blood sample of 4.5 ml was withdrawn from each patient and was immediately transferred to conical tubes containing 0.5 ml trisodium citrate as an anticoagulant, making 1:9 anticoagulant to blood ratio.⁹ Body mass index was expressed as weight (Kg)/height (m²). On the basis of cut-off values for obesity in Asian population, the patients were categorized as underweight with BMI < 18.5 Kg/m², normal weight with BMI 18.5–22.99 Kg/m², overweight with BMI 23–24.99 Kg/m², and obese with BMI ≥ 25 Kg/m².¹⁰

Platelet aggregation studies were performed using Chronolog Light Transmission Aggregometer (Chrono-Log two chamber 490 Model, Chrono-Log Corporation, Havertown, Pennsylvania, USA). Blood samples were centrifuged at 800 rpm for 10 minutes resulting in formation of platelet rich plasma (PRP), 500 µL of which was taken into Chronolog cuvette placed in test chamber of aggregometer. The remaining blood sample was again centrifuged at 4,000 rpm for 5 min, resulting in formation of platelet poor plasma (PPP), 500 µL of which was taken into Chronolog cuvette placed in reference chamber. This PPP was used as reference. The PRP was stirred with the help of siliconized micro stirrer at 1,000 rpm. Before adding agonist, PRP transmits 0% light because medium is full of platelets, while PPP transmits 100% light as it lacks platelets. Adenine diphosphate (5 µL) was used to stimulate platelets and added to 500 µL PRP to make final concentration of 10 µM. The test was run for 3–5 min. Transmission of light through PRP was compared to baseline and PPP and plotted on graph paper by computer using AGGRO/LINK[®] Opti8[™] Software.^{8,11}

Clopidogrel resistance is persistent platelet aggregation despite adequate antiplatelet treatment.¹² On the basis of optical aggregometry, patients having platelet aggregation of < 50% were classified as 'clopidogrel responders' and patients with ≥ 50% aggregation were classified as 'clopidogrel resistant'.^{13,14}

The data was analyzed using Microsoft Excel 2016 and SPSS-23. Continuous data was represented as Mean±SD. Independent sample *t*-test was applied to compare the means of two independent groups. Chi square test was applied to compare frequency distributions of categorical variables among two or more independent groups, and $p \leq 0.05$ was considered significant.

RESULTS

There were 232 (59.50%) males and 158 (40.50%) females with mean age of 53.50±11.87 years. Table-1 is a cross-tabulation between clopidogrel response status and different BMI categories showing frequency and percentage of clopidogrel responders and clopidogrel resistant patients in each BMI group. Chi-square test revealed that there was significant difference ($p < 0.02$) between clopidogrel responder and clopidogrel resistant patients in different BMI groups.

The *t*-test was applied to compare the mean BMI of clopidogrel responders and clopidogrel resistant patients. Table-2 illustrates that mean BMI of the clopidogrel responders was significantly lower than the mean BMI of clopidogrel resistant patients. Figure-1 illustrates the correlation between BMI and platelet aggregation along with values for correlation coefficient and alpha error.

Table-1: Frequencies and percentages of clopidogrel responders and clopidogrel resistant patients in different BMI groups

BMI Groups Kg/m ²	Clopidogrel responders		Clopidogrel resistant	
	N	%	N	%
<18.5	7	100	0	0
18.5–22.99	128	80.5	31	19.5
23–24.99	75	75.8	24	24.2
≥25	83	66.4	42	33.6
<i>p</i>	0.019			

Table-2: Mean BMI of clopidogrel responders and clopidogrel resistant patients

Clopidogrel response status	BMI (Mean±SD) Kg/m ²	<i>p</i>
Clopidogrel responder	24.15±3.39	<0.001
Clopidogrel resistant	26.46±3.82	
Total	24.73±3.63	

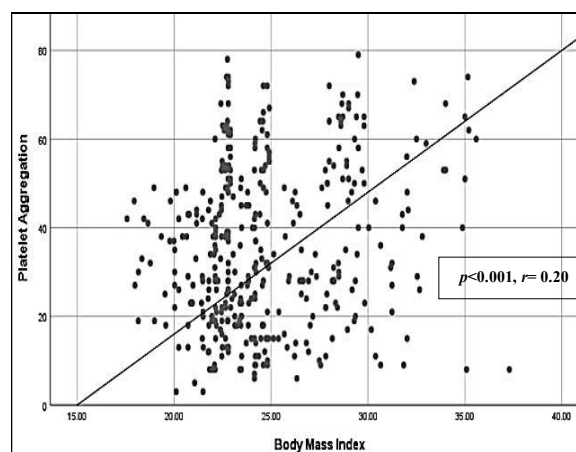


Figure-1: Scatter plot showing correlation between BMI and platelet aggregation

DISCUSSION

We found that frequency of clopidogrel resistance increased progressively in patients with higher BMI.

Maximum number of clopidogrel responders was in normal weight category followed by overweight and obese patients, while maximum number of clopidogrel resistant patients was in obese category followed by overweight and normal categories. No resistance was found in underweight patients though the number of patients was very less. Recently, Peng *et al*¹⁵ conducted a study on patients taking clopidogrel for ischemic stroke and transient ischemic attack. They found that patients with clopidogrel resistance had higher serum cholesterol level and higher BMI.¹⁵

We found that mean BMI of clopidogrel responder patients was significantly lower than mean BMI of clopidogrel resistant patients. Feher *et al*¹⁶ also found that the mean BMI of clopidogrel responder patients was significantly lower than the mean BMI of clopidogrel resistant patients. Presence of diabetes mellitus along with higher BMI has been associated with increased incidence of clopidogrel resistance. Wang *et al*¹⁷ found that mean BMI of clopidogrel responders patients was significantly lower than clopidogrel resistant patients.

Mechanisms related to obesity augmenting platelet aggregation and decreasing efficacy of clopidogrel are complex. Wu *et al*³ proposed obesity as independent risk factor for clopidogrel resistance. They suggested that insulin resistance in type 2 diabetes and obesity could be a cause of upregulation of ADP P2Y₁₂ receptor, which is target site of clopidogrel, hence leading to clopidogrel resistance. Obesity along with hyperglycemia, hypertriglyceridemia, or reduced HDL-C and elevated blood pressure can lead to increased production of clotting factors, and reduction in fibrinolysis. Along with this, dyslipidemia and endothelial dysfunction augment platelet aggregation, increasing the risk of thrombotic events in all types of vessels. Such hypercoagulable and platelet hyperactivity state inside the body may be a reason for decrease clopidogrel efficacy in terms of decreased platelet inhibition.^{18,19}

Anfossi *et al*²⁰ proposed that obesity is associated with tissue resistance to insulin and other mediators acting via intracellular substances like nitrates and prostacyclin. It can lead to disturbance in intracellular ionic balance leading to elevated intracellular calcium level, increased oxidative stress producing isoprostane from arachidonic acid leading to platelet hyperactivity. Such factors lead to the reduced sensitivity of platelets to the physiological and pharmacological antiplatelet agents like clopidogrel.²⁰ Leptin is a hormone which is important in the pathogenesis of obesity. This leptin has been anticipated to regulate hemostasis and play a role in atherosclerotic events in the body by increasing platelet aggregation. Platelets in obese people are hypersensitive to minor quantities of agonists in blood.²¹

Davi *et al*²² observed that obesity is associated with low grade inflammatory state which through enhanced lipid peroxidation leads to thromboxane induced platelet activation. Other factors such as proinflammatory effects of C-reactive protein, release of inflammatory cytokines from activated platelets and F₂-isoprostane induced gene expression of inflammatory cytokines, also lead to amplified platelet activation.²²

Resistance to clopidogrel in obese patients can be overcome by decreasing body weight or increasing the maintenance dose of clopidogrel from 75 mg/day to 150 mg/day or replacing clopidogrel with other ADP antagonist like ticagrelor, but detailed studies in this regard are lacking to provide any confirmed evidence.³ Increasing dose of clopidogrel in obese patients or replacing it with other drugs is a potential scope of future studies.

CONCLUSION

High BMI is related to clopidogrel resistance and it should be considered as independent risk factor for clopidogrel resistance.

REFERENCES

1. Bashir S, Poomima R. Pharmacogenetic variations related to clopidogrel resistance and its clinical implications: An issue which remains largely unaddressed. *Asian J Pharm Clin Res* 2016;9(5):194-6.
2. Gallotta A, Marengoni A, Pasina L, Cortesi L, Nobili A. Prevalence of older in-patients at risk of clopidogrel resistance according to the STIB score. Results from REPOSI registry. *Eur J Intern Med* 2017;41:e17-8.
3. Wu ZK, Wang JJ, Wang T, Zhu SS, Chen XL, Liu C, *et al*. Clopidogrel resistance response in patients with coronary artery disease and metabolic syndrome: the role of hyperglycemia and obesity. *J Geriatr Cardiol* 2015;12(4):378-82.
4. Wang ZJ, Zhou YJ, Liu YY, Yu M, Shi DM, Zhao YX, *et al*. Impact of clopidogrel resistance on thrombotic events after percutaneous coronary intervention with drug-eluting stent. *Thromb Res* 2009;124(1):46-51.
5. Cui G, Zhang S, Zou J, Chen Y, Chen H. P2Y₁₂ receptor gene polymorphism and the risk of resistance to clopidogrel: A meta-analysis and review of the literature. *Adv Clin Exp Med* 2017;26(2):343-9.
6. Bonello-Palot N, Armero S, Paganelli F, Mancini J, De Labriolle A, Bonello C, *et al*. Relation of body mass index to high on-treatment platelet reactivity and of failed clopidogrel dose adjustment according to platelet reactivity monitoring in patients undergoing percutaneous coronary intervention. *Am J Cardiol* 2009;104:1511-5.
7. Al-Azzam SI, Alzoubi KH, Khabour OF, Nusair MB, Al-Hadidi H, Awidi A, *et al*. Factors that contribute to clopidogrel resistance in cardiovascular disease patients: environmental and genetic approach. *Int J Clin Pharmacol Ther* 2013;51(3):179-86.
8. Shetkar SS, Ramakrishnan S, Seth S, Chandna P, Verma SK, Bhargava B, *et al*. CYP 450 2C19 polymorphisms in Indian patients with coronary artery disease. *Indian Heart J* 2014;66(1):16-24.
9. Shalia KK, Shah VK, Pawar P, Divekar SS, Payannavar S. Polymorphisms of MDR1, CYP2C19 and P2Y₁₂ genes in Indian population: Effects on clopidogrel response. *Indian Heart J* 2013;65(2):158-67.
10. Humayun A, Shah AS, Alam S, Hussein H. Relationship of body mass index and dyslipidemia in different age groups of male and

- female population of Peshawar. *J Ayub Med Coll Abbottabad* 2009;21(2):141–4.
11. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Sabaté M, Jimenez-Quevedo P, *et al.* Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. *Diabetes* 2005;54:2430–5.
 12. Gurbel PA, Tantry US. Clopidogrel resistance? *Thromb Res* 2007;120(3):311–21.
 13. Kumar S, Saran RK, Puri A, Gupta N, Sethi R, Surin WR, *et al.* Profile and prevalence of clopidogrel resistance in patients of acute coronary syndrome. *Indian Heart J* 2006;59(2):152–6.
 14. Bliden KP, DiChiara J, Tantry US, Bassi AK, Chaganti SK, Gurbel PA. Increased risk in patients with high platelet aggregation receiving chronic clopidogrel therapy undergoing percutaneous coronary intervention. *J Am Coll Cardiol* 2007;49(6):657–66.
 15. Peng Fei, Xing Yan-hui MS. Analysis of clopidogrel resistance and its influencing factors in patients with ischemic stroke and transient ischemic attack. *Chinese J Clin Neurosci* 2017;3:10.
 16. Feher G, Koltai K, Alkonyi B, Papp E, Keszthelyi Z, Kesmarky G, *et al.* Clopidogrel resistance: role of body mass and concomitant medications. *Int J Cardiol* 2007;120(2):188–92.
 17. Wang L, Wang X, Chen F. Clopidogrel resistance is associated with long-term thrombotic events in patients implanted with drug-eluting stents. *Drugs in R & D* 2010;10(4):219–24.
 18. Dentali F, Squizzato A, Ageno W. The metabolic syndrome as a risk factor for venous and arterial thrombosis. *Semin Thromb Hemost* 2009;35(5):451–7.
 19. Nieuwdorp M, Stroes ES, Meijers JC, Büller H. Hypercoagulability in the metabolic syndrome. *Curr Opin Pharmacol* 2005;5(2):155–9.
 20. Anfossi G, Russo I, Trovati M. Platelet dysfunction in central obesity. *Nutr Metab Cardiovasc Dis* 2009;19(6):440–9.
 21. Konstantinides S, Schäfer K, Koschnick S, Loskutoff DJ. Leptin-dependent platelet aggregation and arterial thrombosis suggests a mechanism for atherothrombotic disease in obesity. *J Clin Invest* 2001;108(10):1533–40.
 22. Davi G, Guagnano MT, Ciabattini G, Basili S, Falco A, Marinopicoli M, *et al.* Platelet activation in obese women: role of inflammation and oxidant stress. *JAMA* 2002;288:2008–14.

Address for Correspondence:

Dr. Usman Nawaz, Department of Pharmacology & Therapeutics, Army Medical College, National University of Medical Science, Rawalpindi, Pakistan. **Cell:** +92-333-5641664

Email: drusmannawaz@gmail.com

Received: 7 May 2018

Reviewed: 4 June 2018

Accepted: 5 June 2018